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Functional Gene Composition of Soil Microbial Communities Across a Latitudinal Gradient in the Arctic Region

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Abstract: Here, we report the investigation of soil microbial community functional genes present in sub-, low-, and high-Arctic regions detected using the GeoChip 5.0M microarray. Predominant functional genes detected in all the soil samples are involved in metal homeostasis, stress response, and carbon cycling.

1. Background & Summary

In an effort to understand the microbial distribution in the Arctic region, most studies focused on the amplicon sequencing of the microbe-specific gene, especially the 16S rRNA gene[1,2]. In comparison, the study of the functions of these microbial community is still relatively lacking. Arctic microorganisms play an important role in biogeochemical cycling of materials such as carbon[3], methane[4] and nitrogen[5]. Warming of the Arctic region often brought upon changes to the Arctic landscapes such as vegetation line expansion[6] and thawing of permafrost[7], which in turn, alters the microbial composition and function. Hence, assessing and understanding the microbial functions in different Arctic regions are crucial to predict how these microorganisms will potentially alter the ecosystem functions as the Arctic warms. Here, we investigated the soil microbial functions from three different habitats in the Arctic region using the high throughput microarray, GeoChip 5.0M. GeoChip 5.0M is one of the most powerful functional gene microarray containing ~162,000

oligonucleotide probes spanning over 1500 gene families involved in biogeochemical processes such as carbon, nitrogen, phosphorus, sulfur cycling, and six other major gene categories[8]. All the functional gene categories covered by GeoChip 5.0M were described previously[8].

The number of functional genes detected by GeoChip 5.0M from the DNA of soil samples collected from Whapmagoostui-Kuujjuarapik (KW), Salluit, and Pond Inlet (Figure 1) were 89,111, 90,811, and 91,610 genes, respectively. A total of 60,056 genes, 57,891 genes, and 54,726 genes were detected from the cDNA of soil samples collected from KW, Salluit, and Pond Inlet, respectively. A higher number of genes were detected from the soil DNA samples compared with the soil cDNA samples, suggesting that only a fraction of the genes present in the Arctic environment (DNA samples) were actively expressed (cDNA samples). Most of the functional genes detected from the DNA and cDNA samples originated from bacteria, but genes originating from eukaryotes and viruses were also detected (Figure 2). Predominant functional genes detected from the soil samples were primarily related to metal homeostasis, stress response, carbon cycling, antibiotic resistance, and organic contaminant degradation (Figure 3). To our knowledge, this is the first systematic study on the functional gene composition in the high-, sub- and low- Canadian Arctic regions. The data from this study can provide valuable baseline information on the functional gene pool in these areas.

2. Location

Sampling was performed at the Arctic region of KW (Sub-Arctic, 55.3°N 77.8°W), Salluit (Low-Arctic, 62.1°N 75.4°W) and Pond Inlet (High Arctic, 52.5°N 77.6°W) in October 2018 (KW), June 2017 (Salluit) and August 2018 (Pond Inlet). The annual mean air temperature in KW, Salluit and Pond Inlet are approximately –4.0 °C, –8.5 °C and –14.4 °C, respectively[9]. A detailed map of the sampling areas can be found in Figure 1. These sites are located along eastern Hudson Bay or Baffin Bay. Salluit and Pond Inlet have glacial and periglacial landforms, while KW consists of typical landscapes on the Canadian shield. The main vegetation types are erect dwarf shrub tundra in KW; graminoid, prostrate dwarf-shrub, forb tundra in Salluit; Nontussock sedge, dwarf-shrub, moss tundra on Pond Inlet[10]. Detailed descriptions of the sampling sites were outlined in previously published papers[11–14].

3. Methods

3.1. Soil Sampling

Soil samples were chosen from five random sampling points at each sampling area and were collected using a sterile scoop and placed into a 5-mL sampling tube containing RNA*later* Stabilization Solution (Ambion, Austin, Texas, USA). The samples were frozen at −20°C immediately and shipped to the National Institute of Polar Research, Tokyo, Japan in frozen state for

further analyses. Total DNA and RNA were extracted from each soil sample using ZymoBIOMICS DNA & RNA kit (Zymo Research, Irvine, CA, USA). Total RNA extracted was subjected to DNAse I treatment. Success of the DNase treatment was checked by no PCR amplification of the V1-V3 Bacterial 16S rRNA gene. The cDNA was generated from the RNA template using SuperScript IV First-Strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA, USA), in accordance with the manufacturer's instructions.

3.2. DNA Microarray Hybridization and Data Processing

For each soil sample, the DNA and its paired cDNA samples were sent to Glomics Inc., Oklahoma, USA) for functional gene sequencing using the GeoChip 5.0M Microarray manufactured by Agilent (Agilent Technologies Inc., Santa Clara, USA)[8]. 500 ng of DNA was labeled with the fluorescent dye Cy-3 (GE Healthcare, CA, USA) by random priming with Klenow fragment, cleaned using a QIAquick purification kit (Qiagen), and then dried. Labeled DNA was suspended in hybridization solution containing 10 % formamide, Hi-RPM Hybridization Buffer, aCGH blocking agent, Cot-1 DNA, and common oligonucleotide reference standards. Then the solution was denatured at 95 °C for 3 min, incubated at 37 °C for 30 min, loaded to the microarray slide well, and hybridized at 67 °C for 24 h. After hybridization, slides were rinsed and imaged with a NimbleGen MS200 microarray scanner (Roche NimbleGen, Madison, WI, USA). Functional gene names listed in this study were assigned in accordance with the original functional gene annotations in GeoChip 5.0M (http://ieg.ou.edu/gcs/gcsmm.cgi?version=gc50_180k).

4. Data Records

All raw and processed GeoChip 5.0M data were deposited to NCBI Gene Expression Omnibus (GEO, https://www.ncbi.nlm.nih.gov/geo/) under the accession number GSE168911 for KW, GSE168623 for Salluit and GSE168985 for Pond Inlet.

5. Technical Validation

To call the GeoChip 5.0M probes positive, a floating signal-to-noise ratio (SNR) were applied so that the hyperthermophile probes accounted for 5 % of the positive signals. Probes were deemed negative if the SNR of the probe was less than 2, the signal value was less than 200 or less than 1.3 times the background. Negative probes were removed from the downstream analyses. Additionally, the signal intensity of each spot was normalized by mean and outlier were removed if any of replicate slides had (signal–mean) more than three times the standard deviation.

6. Usage Notes

Requests for permission to reuse the content (raw data) from this paper can be submitted in writing to the following email address: uchida@nipr.ac.jp.

8. Figures

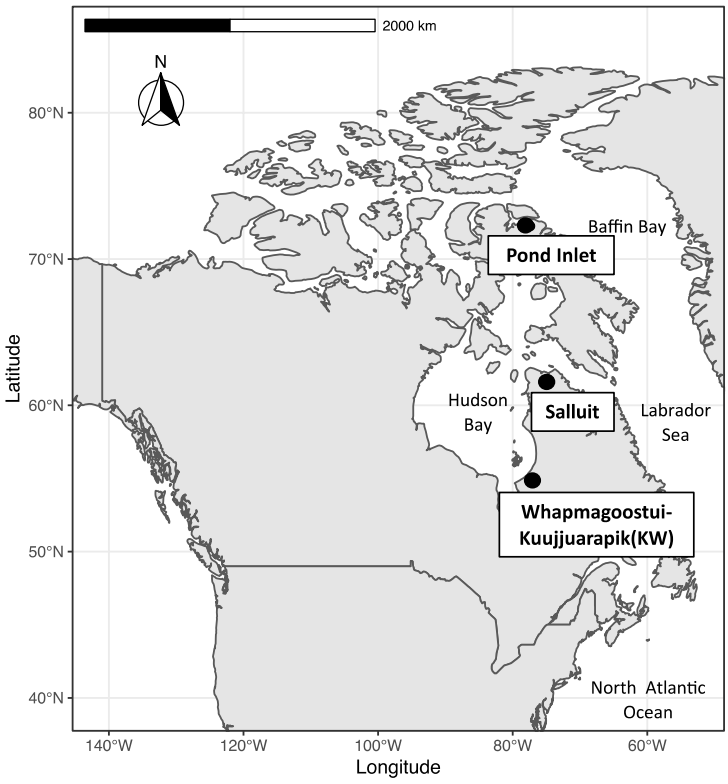

Figure 1. Sampling sites at mid-Arctic (Whapmagoostui-Kuujjuarapik, KW), low-Arctic (Salluit) and high-Arctic (Pond Inlet).

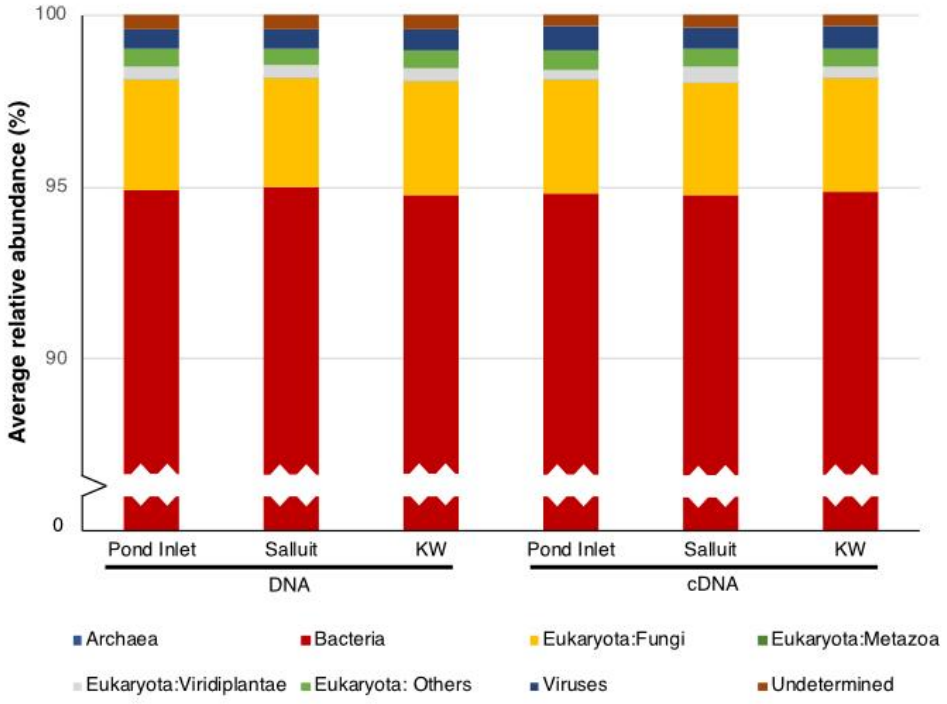

Figure 2. The phylogenetic distribution of functional genes detected by GeoChip 5.0M. The average relative abundance was calculated from five soil samples (DNA: n=5, cDNA: n=5) collected at each sampling area.

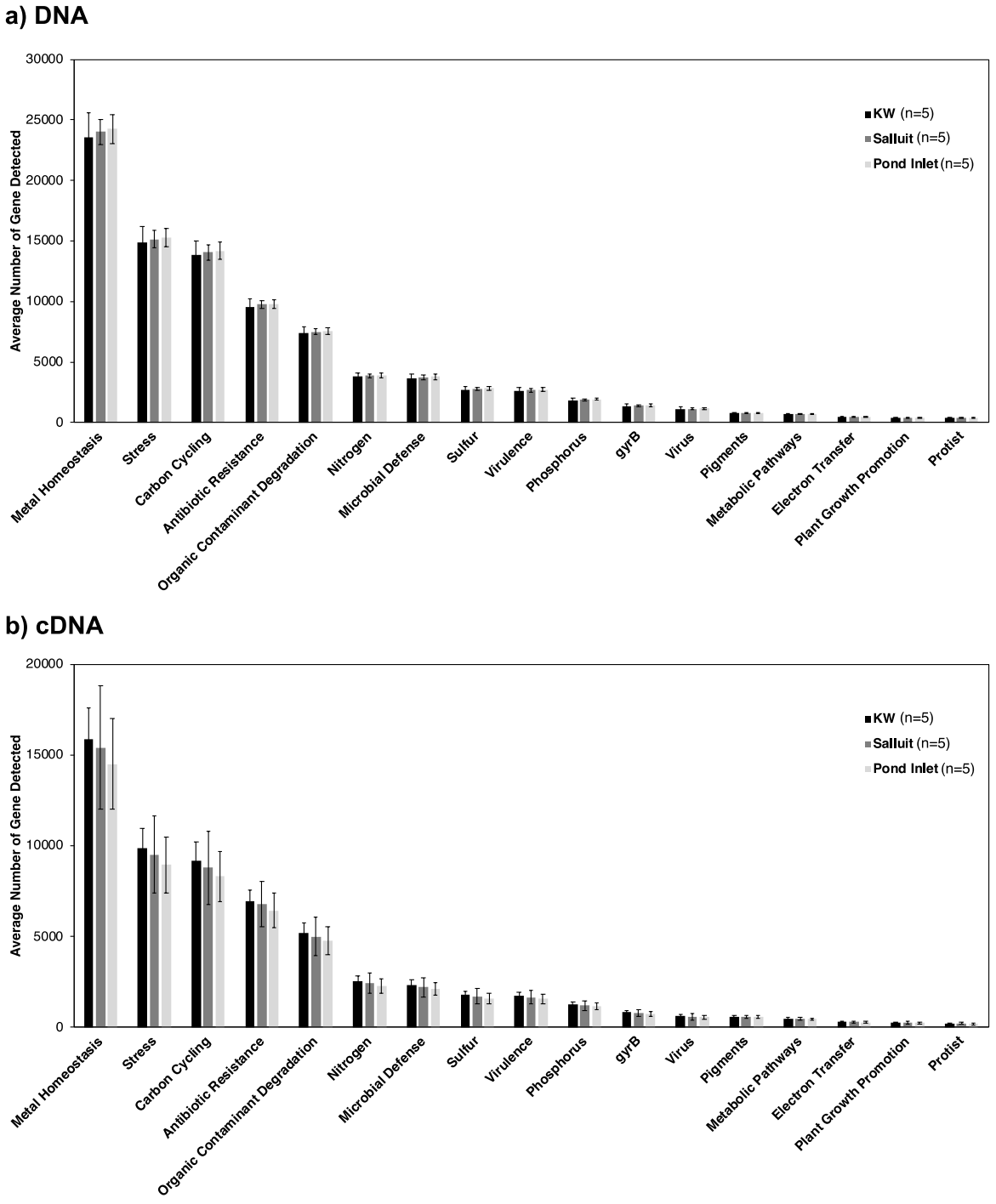

Figure 3. Average number of genes detected from each GeoChip 5.0M functional gene categories from a) DNA samples and b) cDNA samples. Error bars represent standard deviation.

Author contributions

M. Uchida and A.S. Mori conceived and designed the study. R. Kaneko, S. Masumoto and R. Kitagawa collected and processed samples. S-K Wong performed bioinformatics processing and drafted the manuscript. All authors were involved in the critical review and final drafting of the manuscript. All authors read and approved the final manuscript.

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Data Citation

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